
A New Species of *Leymus* (Poaceae, Triticeae) from China

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ABSTRACT. A new species of *Leymus* Hochst. (Poaceae, Triticeae) from Inner Mongolia, China, *L. pubens* H. X. Xiao, is described, illustrated, and compared with its closest relatives. This species was initially identified as *L. chinensis* (Trin.) Tzvelev, but the upper surfaces of its leaf blades are spinulose, and the internode below the spike, upper half of the rachis, rachilla, and lemmas are sparsely puberulent. A key is provided for *L. pubens* and closely related species of *Leymus*. The identification of the new species is also supported by nuclear ribosomal ITS and chloroplast *trnL-F* sequence analyses.

Key words: China, Inner Mongolia, ITS, IUCN Red List, *Leymus*, Poaceae, Triticeae, *trnL-F*.

Leymus Hochst. is an important perennial genus in the wheat tribe (Poaceae, Triticeae). Hochstetter (1848) separated it from traditional *Elymus* L., with *L. arenarius* (L.) Hochst. as the type species. *Leymus* includes ca. 63 species and 19 subspecies that are distributed in diverse ecological habitats across temperate regions of Eurasia, North America, and South America, also extending into subtropical and tropical alpine regions (Yen et al., 2009). *Leymus* species grow in saline or alkaline soils, from dry or semi-dry areas, and are well adapted to cold and dry conditions.

During fieldwork on the Songnen steppe in July 2009, several interesting plants initially identified as *Leymus chinensis* (Trin.) Tzvelev were collected. At first sight, the taxon appeared similar to *L. chinensis* yellow-green type (yellow-green leaf blades). However, this collection exhibited several characters distinct from typical *L. chinensis*. On subsequent visits to this and adjacent localities in June of 2010, additional material was collected, including fertile spikes. Preliminary work at HNWP and NENU suggested a distinctive and new species, which is presented in this paper. In addition, based on taxonomic literature and expert specialists (Cai, 2000; Zhi & Teng, 2005; Chen & Zhu, 2006; Yen et al., 2009) in the field, our material differed from any currently recognized species of *Leymus*. ITS and chloroplast *trnL-F* sequences analysis further supported the taxon's recognition as a new species.

Sequences from the nuclear ribosomal ITS and chloroplast *trnL-F* genic region were used, based on their relevance for phylogenetic reconstruction in angiosperms at lower taxonomic levels (Baldwin et al., 1995; Hodkinson et al., 2007).

MATERIALS AND METHODS

MORPHOLOGICAL ANALYSIS

Morphological and morphometrical studies were carried out on specimens, with comparisons made to the materials kept at HNWP and NENU, and also to the taxonomic literature such as *Flora Europaea* (Melderis, 1980), *Flora Qinghaiica* (Cai, 1999), *Flora Xinjiangensis* (Cui, 1996), and *Flora Intramongolica* (Yang, 1994). At least 30 measurements on fresh material were performed on each morphological variable by using a stereoscopic microscope (Motic K-400L; Motic, Xiamen, China) with a digital camera. The following parameters were selected to describe the morphology of the studied specimens: ligule lengths, leaf blades, rachis internodes, spike morphology, pubescence, glume and lemma shapes, etc. Microphotographs were taken with a Moticam 2506 camera (Motic) and then processed with Image Pro-Plus software version 4.5 (Media Cybernetics, Inc., Silver Springs, Maryland, U.S.A.).

PLANT MATERIAL

Fifteen accessions of *Leymus*, including the new species, were sequenced for nuclear ribosomal ITS and chloroplast *trnL-F* in the present study, with 12 accessions identified as the *L. chinensis* gray-green type (six accessions) and *L. chinensis* yellow-green type (six accessions). Each taxon was collected from three different regions across the distributional range of the suspected new species, according to soil type. The sequences generated in this study were analyzed together with 12 related Triticeae using *Bromus catharticus* Vahl as the outgroup, based on its use as an outgroup by previous phylogenetic studies of the Poaceae (e.g., Bouchenak-Khelladi et al., 2008; Sha et al., 2008). GenBank accession numbers and voucher details are listed in Table 1.

DNA EXTRACTION, POLYMERASE CHAIN REACTION AMPLIFICATION, AND SEQUENCING

Genomic DNA was extracted from ca. 50 mg of fresh or 10 mg silica-dried leaf materials using the E.Z.N.A. Plant DNA Mini Kit (Omega Bio-tek, Inc., Norcross, Georgia, U.S.A.), according to the manufacturer's instructions. The entire ITS region (ITS-1, 5.8S ribosomal DNA, and ITS-2) was amplified by using primers ITS-1 and ITS-4 (White et al., 1990), while the chloroplast *trnL-F* was amplified by using primers *c* and *f* (Taberlet et al., 1991). An Applied Biosystems (Carlsbad, California, U.S.A.) 2720 PCR System was used, with cycling as follows: 94° for 5 min., 35 cycles at 94° for 1 min., 53° for 1 min., 72° for 1 min., followed by 72° for 10 min. The cloned PCR products were sequenced in both directions by Sangon Co., Ltd. (Shanghai, China), and all the sequences used in the phylogenetic analysis were derived from at least five independent clones to examine whether there were multiple copies within individuals (intra-individual polymorphisms). Forward and reverse sequences of each sample were manually edited and combined into a single consensus sequence.

PHYLOGENETIC ANALYSIS

Sequences of the nuclear ribosomal ITS and chloroplast *trnL-F* were aligned with ClustalX version 2 (Larkin et al., 2007) and adjusted manually. Phylogenetic trees were constructed by using the neighbor-joining algorithms available in MEGA version 3.1 (Kumar et al., 2004). The topology of the tree was evaluated by using Kimura's two-parameter calculation model. Gaps were coded as missing data with the pairwise deletion option, and for testing the robustness of the clades, a bootstrap analysis was performed with 1000 replicates (Liu et al., 2008).

RESULTS

MORPHOLOGY

The new species was initially identified as *Leymus chinensis*, based on its similar, yellowish green leaf blades. However, the new species may be contrasted by its spinulate adaxial leaf blades and the sparse puberulence of the upper half of the rachis and rachilla, as well as the internode immediately below the spike and lemmas. In contrast, adaxial leaf blades of *L. chinensis* are puberulent, and the rachis, rachilla, and internode immediately below the spike as well as the lemmas are glabrous (Fig. 1). The new species also resembles *L. karelinii* (Turcz.) Tzvelev and *L. angustus* (Trin.) Pilg. in that the lemmas and the

upper portion of the rachis are dorsally puberulent. The new species differs from both taxa by its creeping rhizomes and spikelets with four to eight florets, while *L. karelinii* and *L. angustus* have only downward rhizomes and spikelets that are 2- to 3-flowered.

PHYLOGENETIC ANALYSIS

In the neighbor-joining dendrogram, 14 *Leymus* accessions resolved into two principal groups (Fig. 2). All representatives of the new species and all 12 for *L. chinensis* were in group A, which was composed of four subclades, with one including the *L. chinensis*–*L. pubens* clade. Within this subclade, 15 accessions of *L. chinensis* and *L. pubens* comprised monophyletic groups, with 99% bootstrap support, respectively. Bootstrap values higher than 70% would indicate these clades as reliable. Each individual accession in the two groups was gathered from different distributional locales. The *L. chinensis*–*L. pubens* clade resolved as sister to a clade including *L. arenarius*, *L. secalinus* (Georgi) Tzvelev, *L. pseudoracemosus* C. Yen & J. L. Yang, *L. mollis* (Trin.) Pilg., *L. karelinii* (Turcz.) Tzvelev, *L. angustus* (Trin.) Pilg., *L. akmolinensis* (Drobow) Tzvelev, and *L. racemosus* (Lam.) Tzvelev, all morphologically distinct from species in the sister clade. The three accessions of *L. pubens* showed nucleotide differences in the ITS region (five substitutions) and chloroplast *trnL-F* (14 substitutions), with respect to *L. chinensis*. These molecular differences further support the taxonomic separation of the two species.

***Leymus pubens* H. X. Xiao, sp. nov. TYPE: China.**

Inner Mongolia: Tongliao, Liangzhijian, on sandy places, 44°26.633'N, 122°02.581'E, 165 m, 10 June 2010, H. X. Xiao 40003 (holotype, NENU; isotypes, NENU, National History Museum of Jilin). Figure 3.

Haec species *Leymo chinensi* (Trin.) Tzvelev similis, sed ab ao lamina foliari adaxialiter spinulosa atque rhachidis internodio infra spicam ac dimidio superiore sicut rhachilla lemmatibusque omnibus parce puberulis distinguitur.

Perennial herbs, yellow-green, with long rhizomes creeping and downward; culms solitary or caespitose, 30–90 cm tall, with 3 to 4 nodes. Leaf sheaths glabrous, the basal sheaths persistent, sometimes disintegrating into fibers; ligules papery, 1.5–2(2.5) mm long, obtuse; leaf blades involute, 5–25 × 3–5 cm, upper surfaces and the margins spinulate, lower surfaces glabrous. Spikes lax, 7–20 cm; rachis internodes generally 5–11 mm, or basal ones up to 30 mm, dorsally puberulent in upper portion, margins ciliate. Spikelets usually paired, rarely one at each

Table 1. Sources of plant material used in this study. The accession numbers correspond to the numbers used in Figure 2. One asterisk denotes that the voucher specimen was deposited at the herbarium of the School of Life Sciences, Northeast Normal University, China (NENU). Two asterisks indicate that the sequences were taken from GenBank (<<http://www.ncbi.nlm.nih.gov/genbank/>>). NA refers to data not available. The soil type classification is from previous studies (Li, 2001; Qiu, 2008).

Accession number	Taxon	Voucher	GenBank accession number	
			ITS	<i>trnL-F</i>
1	<i>Leymus chinensis</i> (Trin.) Tzvelev (yellow-green type)	China. Inner Mongolia: Tumuji, Kastanozems soil, 159 m, 11 June 2010, <i>H. X. Xiao 40001*</i>	HQ391341	JQ627774
2	<i>L. chinensis</i> (gray-green type)	China. Inner Mongolia: Tumuji, Kastanozems soil, 159 m, 11 June 2010, <i>H. X. Xiao 40002*</i>	HQ391342	JQ627775
3	<i>L. pubens</i> H. X. Xiao	China. Inner Mongolia: Liangzhijian, Sandy soil, 165 m, 10 June 2010, <i>H. X. Xiao 40003*</i>	HQ391343	JQ627786
4	<i>L. chinensis</i> (gray-green type)	China. Inner Mongolia: Liangzhijian, Sandy soil, 165 m, 10 June 2010, <i>H. X. Xiao 40004*</i>	HQ391344	JQ627776
5	<i>L. pubens</i>	China. Jilin: Changling, Saline-alkali soil, 159 m, 9 June 2010, <i>H. X. Xiao 40005*</i>	HQ391345	JQ627787
6	<i>L. chinensis</i> (gray-green type)	China. Jilin: Changling, Saline-alkali soil, 159 m, 9 June 2010, <i>H. X. Xiao 40006*</i>	HQ391346	JQ627777
7	<i>L. chinensis</i> (yellow-green type)	China. Jilin: Zhenlai, Saline-alkali soil, 146 m, 9 June 2010, <i>H. X. Xiao 40007*</i>	HQ391347	JQ627778
8	<i>L. chinensis</i> (gray-green type)	China. Jilin: Leshan, Black soil, 247 m, 13 June 2010, <i>H. X. Xiao 40008*</i>	HQ391348	JQ627779
9	<i>L. pubens</i>	China. Jilin: Wushiwu, Saline-alkali soil, 146 m, 9 June 2010, <i>H. X. Xiao 40009*</i>	HQ391349	JQ627788
10	<i>L. chinensis</i> (yellow-green type)	China. Jilin: Qianan, Saline-alkali soil, 151 m, 8 June 2010, <i>H. X. Xiao 40010*</i>	HQ391350	JQ627780
11	<i>L. chinensis</i> (yellow-green type)	China. Jilin: Qianguo, Saline-alkali soil, 148 m, 8 June 2010, <i>H. X. Xiao 40011*</i>	HQ391351	JQ627781
12	<i>L. chinensis</i> (gray-green type)	China. Inner Mongolia: Tumuji, Saline-alkali soil, 159 m, 11 June 2010, <i>H. X. Xiao 40012*</i>	HQ391352	JQ627782
13	<i>L. chinensis</i> (yellow-green type)	China. Jilin: Qianguo, Saline-alkali soil, 140 m, 8 June 2010, <i>H. X. Xiao 40013*</i>	HQ391353	JQ627783
14	<i>L. chinensis</i> (gray-green type)	China. Jilin: Qianguo, Saline-alkali soil, 140 m, 8 June 2010, <i>H. X. Xiao 40014*</i>	HQ391354	JQ627784
15	<i>L. chinensis</i> (yellow-green type)	China. Inner Mongolia: Tumuji, Saline-alkali soil, 159 m, 11 June 2010, <i>H. X. Xiao 40015*</i>	HQ391355	JQ627785
16	<i>L. akmolnensis</i> (Drobow) Tzvelev	NA	EF581913**	EF581897**
17	<i>L. angustus</i> (Trin.) Pilg.	NA	EF581920**	EF581909**
18	<i>L. secalinus</i> (Georgi) Tzvelev	NA	EF581975**	EF581904**
19	<i>L. ambiguus</i> (Vasey & Scribn.) D. R. Dewey	NA	EF581916**	EF581905**
20	<i>L. cinereus</i> (Scribn. & Merr.) Á. Löve	NA	EF581946**	EF581899**
21	<i>L. racemosus</i> (Lam.) Tzvelev	NA	EF581968**	EF581903**
22	<i>L. karelinii</i> (Turcz.) Tzvelev	NA	EF581960**	EF581907**
23	<i>L. triticoides</i> (Buckley) Pilg.	NA	EF485567**	EU366404**
24	<i>L. erianthus</i> (Phil.) Dubcovs.	NA	EF601993**	EU366398**
25	<i>L. pseudoracemosus</i> C. Yen & J. L. Yang	NA	EF602016**	EU366399**
26	<i>L. arenarius</i> (L.) Hochst.	NA	EF581934**	EF581906**
27	<i>L. mollis</i> (Trin.) Pilg.	NA	EF581964**	EF581902**
28	<i>Bromus catharticus</i> Vahl	NA	AF521898**	EU036184**

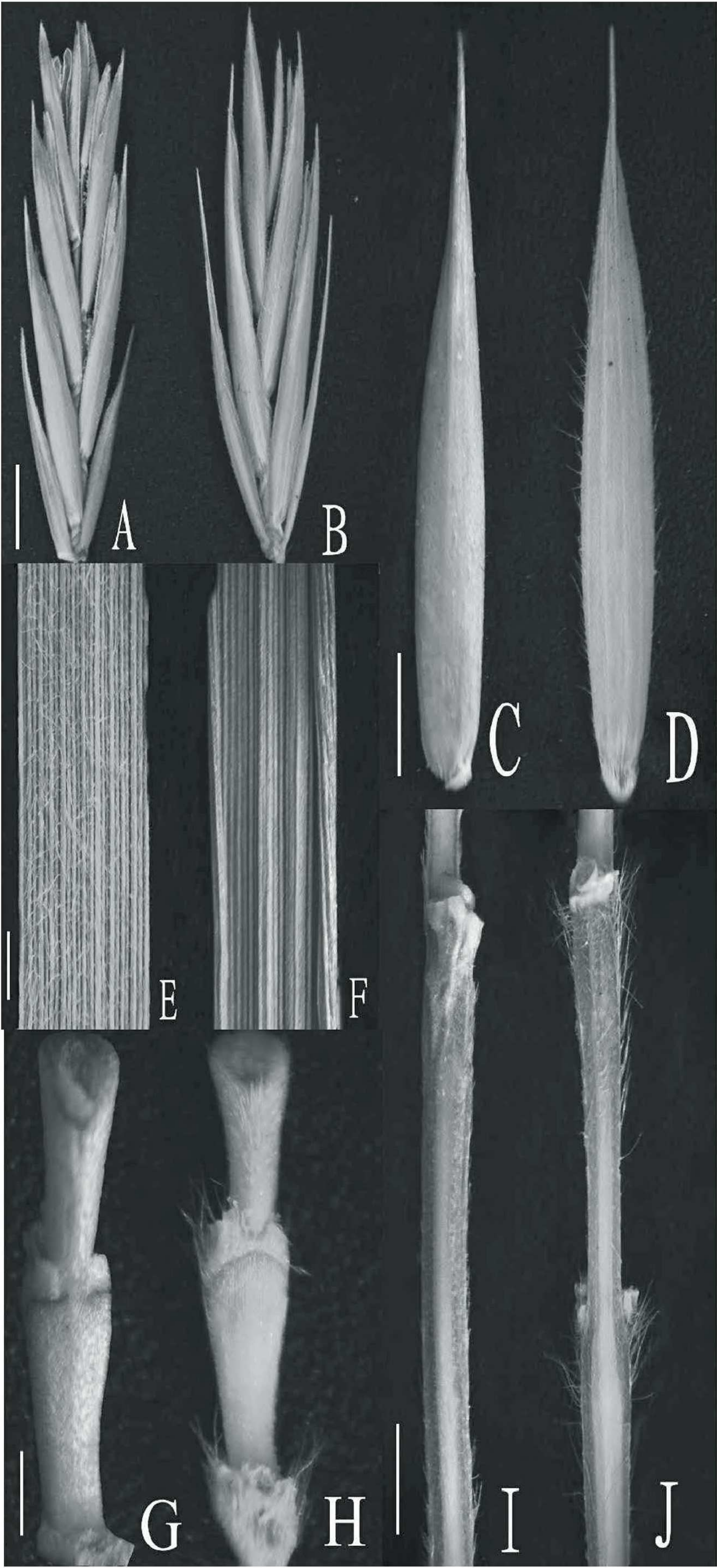


Figure 1. Morphological comparison of the two species of *Leymus* Hochst. A, C, E, G, I. *Leymus chinensis* (Trin.) Tzvelev; B, D, F, H, J. *Leymus pubens* H. X. Xiao. —A, B. Spikelets. —C, D. Glumes. —E, F. Upper surfaces of leaf blade. —G, H. Rachillas. —I, J. Rachises. Scale bars: A, B, E–H = 18 mm; C, D = 9 mm; I, J = 15 mm. Microphotographs taken from *H. X. Xiao 40001* and *H. X. Xiao 40005* (NENU).

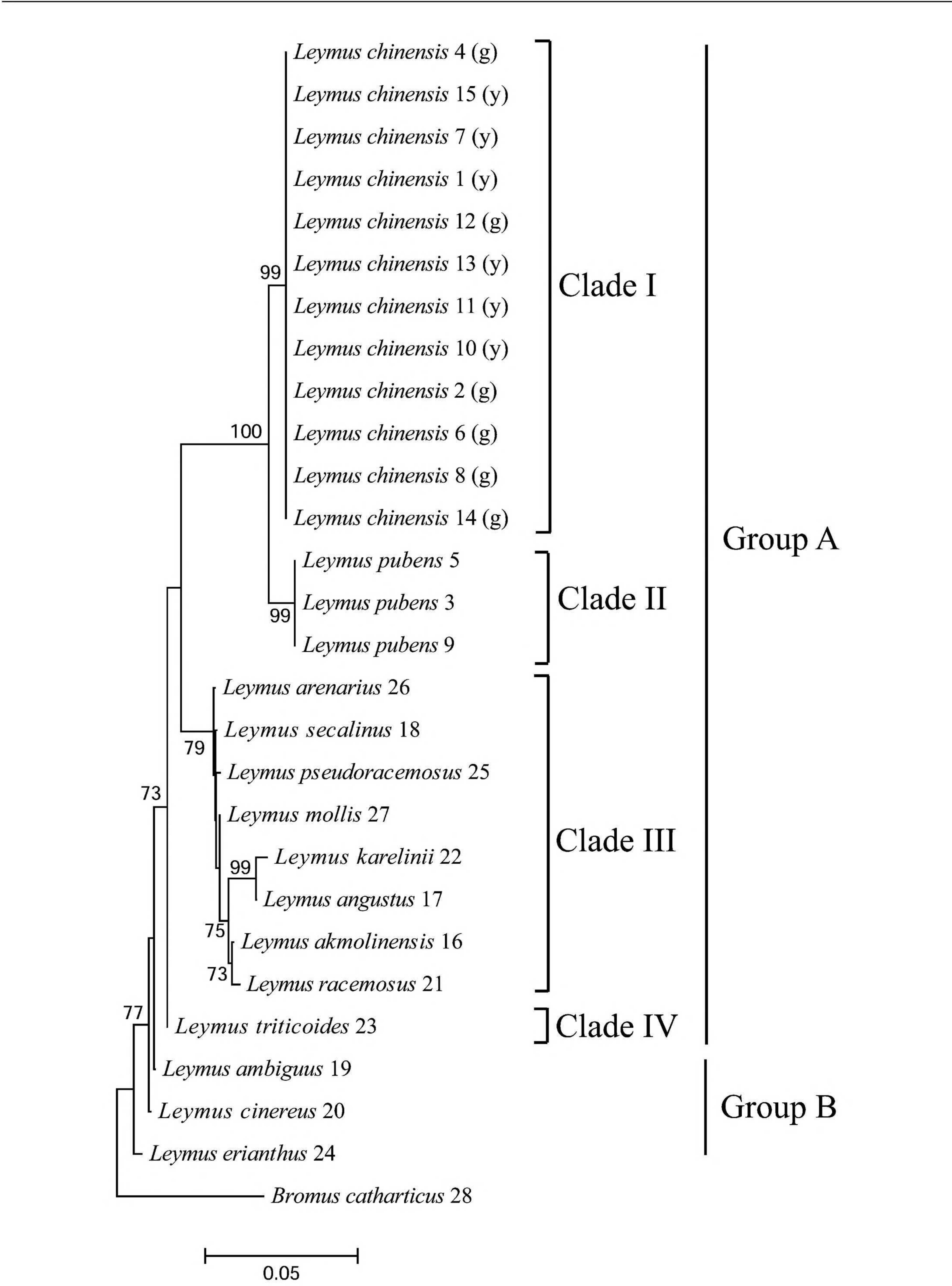


Figure 2. Neighbor-joining tree based on the sequences of nuclear ITS and chloroplast *trnL-F* genic region for *Leymus pubens* H. X. Xiao and related *Leymus* species. Numbers at nodes are bootstrap percentages greater than or equal to 50%. The letter “g” inside parentheses refers to *L. chinensis* (Trin.) Tzvelev gray-green type; the “y” within parentheses refers to *L. chinensis* yellow-green type. Numbers refer to the accessions in Table 1.

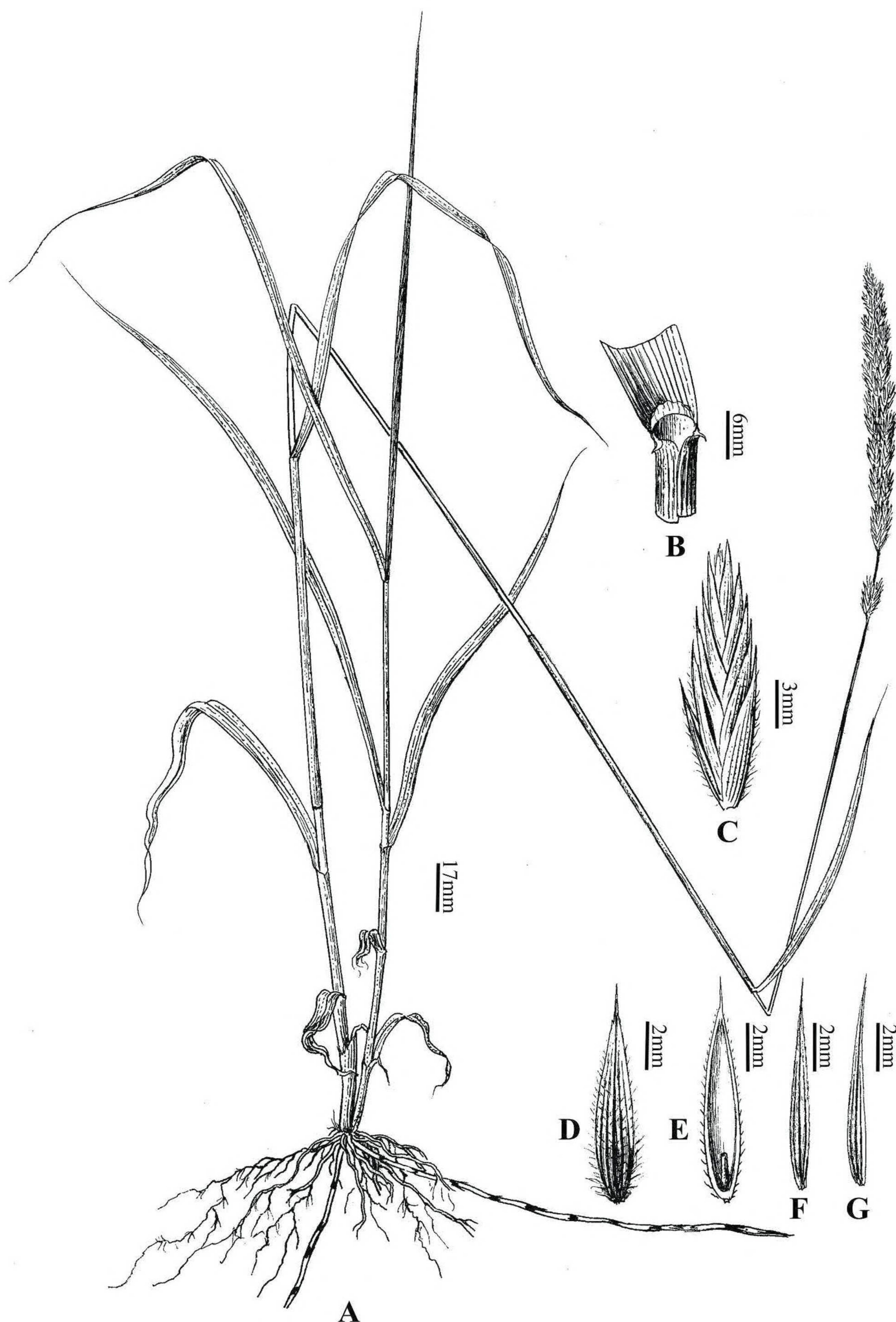


Figure 3. *Leymus pubens* H. X. Xiao. —A. Fertile plant. —B. Junction of sheath and blade to show ligule. —C. Spikelet. —D. Dorsal view of the first floret. —E. Ventral view of the first floret. —F. Lower glume. —G. Upper glume. Drawn by Wu Zhi-xue from the type *H. X. Xiao 40003* (NENU).

node of the rachis, or singly in upper half, 4- to 8-flowered, 10–20 mm long; rachilla internodes 1–3 mm, sparsely puberulent; glumes lanceolate, 1- or 3-nerved, ciliate along the margins, tip with a 2–3(4) mm awn at the apex, first glume 6.5–9.5 mm (excluding awn), second glume 7.5–11 mm; lemmas lanceolate, obscurely 5-nerved, rarely 7-nerved, sparsely puberulent on the back, membranous along the margins, with a short mucro 0.5–2 mm; paleae nearly equal to lemma, bifid, spinulose along the keels; anthers yellowish cream, rarely purplish, ca. 4 mm long; ovary cuneate, densely puberulent, with the trichomes 0.5–0.8 mm long on upper half.

Distribution and habitat. *Leymus pubens* is currently known only from the eastern portion of Inner Mongolia and western Jilin Province in China. The new taxon was observed to grow at the margins of woodlands, grasslands, and roadsides at altitudes from 146 to 165 m on saline-alkali and sandy soil.

IUCN Red List category. *Leymus pubens* is accessed here as Least Concern (LC), according to the IUCN Red List Criteria (IUCN, 2001), based on its relatively extensive distribution. This species is now known only from three different localities of China, spanning a distributional area of approximately 24,000 km². In the area called Changling, this species is relatively more common and the populations seem to have more individuals. However, there is no precise information for the size of the populations, due to the fact that *L. pubens* at first sight is similar to *L. chinensis*, which is a common grass broadly distributed across the Songnen steppe and extending into Inner Mongolia.

Phenology. *Leymus pubens* was observed to flower in May and June, with fruiting in July and August.

Etymology. The specific epithet *pubens* refers to the sparse pubescence on the upper rachis, rachilla, and the stem internode immediately below the spike as well as on the lemmas.

KEY TO SPECIES CLOSELY RELATED TO *LEYMUS PUBENS* IN CHINA

- 1a. Lemmas and upper portion of rachis dorsally puberulent.
 - 2a. Spikelets with 2 or 3 florets, plants with only rhizomes vertically oriented downward.
 - 3a. Lower part of glumes broadly covering the base of the lemma *L. karelinii*
 - 3b. Lower part of glumes narrow, not covering the base of the lemma *L. angustus*
 - 2b. Spikelets with 4 to 8 florets, plants with long rhizomes creeping horizontally and also vertically *L. pubens*

- 1b. Lemmas and upper portion of rachis glabrous
..... *L. chinensis*

Paratypes. CHINA. **Jilin:** Songyuan, Changling, in thick grass on slopes, 159 m, 9 June 2010, H. X. Xiao 40005 (NENU); Songyuan, Wushiwu, 146 m, 9 June 2010, H. X. Xiao 40009 (NENU).

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